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Report

Inbreeding Depression Is Purged in the Invasive Insect *Harmonia axyridis*

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Summary

Bottlenecks in population size reduce genetic diversity and increase inbreeding, which can lead to inbreeding depression [1]. It is thus puzzling how introduced species, which typically pass through bottlenecks, become such successful invaders [2]. However, under certain theoretical conditions, bottlenecks of intermediate size can actually purge the alleles that cause inbreeding depression [3]. Although this process has been confirmed in model laboratory systems [4], it has yet to be observed in natural invasive populations. We evaluate whether such purging could facilitate biological invasions by using the world-wide invasion of the ladybird (or ladybug) *Harmonia axyridis*. We first show that invasive populations endured a bottleneck of intermediate intensity. We then demonstrate that replicate introduced populations experience almost none of the inbreeding depression suffered by native populations. Thus, rather than posing a barrier to invasion as often assumed, bottlenecks, by purging deleterious alleles, can enable the evolution of invaders that maintain high fitness even when inbred.

Results and Discussion

Reductions in population size, or bottlenecks, decrease genetic variation and lead to inbreeding, which can cause inbreeding depression within introduced populations [1]. However, there is growing recognition that the consequences of bottlenecks are varied and that, under some circumstances, they can actually lead to increased individual and population performance [5, 6]. We lack the ability to say whether positive effects of bottlenecks are theoretical curiosities or whether they truly influence the dynamics of natural populations [7]. One mechanism by which bottlenecks can have positive effects is through the purging of deleterious mutations that lead to inbreeding depression [8]. Theory states that for purging to occur, the reduction in population size should be of intermediate intensity (i.e., ranging from 40–300 individuals, depending upon intensity of selection) and the mutations leading to inbreeding depression should be strongly deleterious and highly recessive [3, 7]. Such purging of deleterious mutations has been demonstrated empirically in artificially bottlenecked populations [4, 9, 10], but given the conditions imposed, high rates of extinction have been observed. This makes it difficult to directly extrapolate to natural populations [7]. To date, studies documenting a purge of deleterious mutations during bottlenecks are scarce and rely on indirect evidence [5].

The ability of invasive species to dominate novel ecosystems has been considered puzzling given that they typically pass through bottleneck in population size during introductions ([2], although see [11]. Such bottlenecks have been seen as detrimental to invasion success; the implicit assumption is that they reduce genetic variation, and thereby inhibit the ability of introduced species to adapt to their new environments, and that they increase inbreeding and associated inbreeding depression [2]. However, it may be that rather than increasing inbreeding depression, bottlenecks that occur during invasions tend to be of the intensity that could enhance invasion ability via the purging of the deleterious alleles underlying inbreeding depression.

Here, we use a world-wide invader, the harlequin ladybird *Harmonia axyridis* (HA), as a model system to examine whether bottlenecks might have led to reduced inbreeding depression in invasive populations relative to native ones. Native to Asia, HA was repeatedly introduced as a biological control agent into North America and Europe, but for decades it failed to establish itself. However, by 1988, it had not only established itself in North America but had also rapidly become an invasive pest on a world-wide scale. A recent study showed that invasions of HA followed a bridgehead scenario [12], in which the initial invasive population in eastern North America acted as the source of the invasions into the European, South American, and African continents (Figure 1). This result suggests that an evolutionary shift that triggered invasion probably occurred in the bridgehead population in eastern North America. With this background knowledge, we first use data from neutral genetic markers to test the hypothesis that the introduction of HA in eastern North America was associated with a population bottleneck, and we evaluate whether the size of this bottleneck was of the appropriate level for purging to occur. Then, we experimentally test the hypothesis that invasive populations have evolved reduced inbreeding depression with respect to life-history traits important for invasion success.

We investigated evidence for a bottleneck of an appropriate intensity for purging to occur by using data from 18 microsatellite loci that we analyzed with approximate Bayesian computation [13, 14]. Specifically, we evaluated whether a bottleneck occurred during the introduction of HA from the native area

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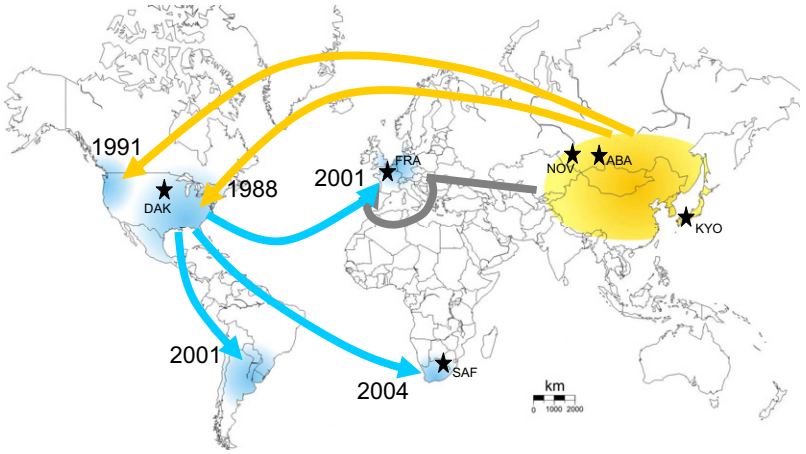

Figure 1. Worldwide Routes of Invasion of *Harmonia axyridis*

For each outbreak, the arrow indicates the most likely invasion pathway [12]. Yellow and blue indicate native and invasive areas, respectively. Years of first observation of invasive populations are indicated. Abbreviations correspond to the samples used in the experimental study (see Experimental Procedures for further explanations).

into the bridgehead population from eastern North America [12], and we estimated its intensity (see Supplemental Experimental Procedures). Two sets of population samples were considered as representative of the native and introduced areas. In one, we used the same populations as those used for the present quantitative genetics studies, and in the other, we used the same populations as those analyzed in [12] to make inferences about introduction routes in *H. axyridis* (see Supplemental Information). We found that a scenario including a bottleneck during the introduction was supported by very high probabilities in comparison to a scenario without a bottleneck (see Supplemental Experimental Procedures). The highest joint posterior densities of the size and duration of the bottleneck corresponded to values around 150 individuals and 20 generations compared to an estimated stable effective population size of several thousand individuals in the native area (Figure 2). Similar results were obtained when we considered other sets of priors and data sets (Figure S1). These results are well within the theoretical range that can lead to the purging of deleterious alleles [3]. However, it has to be noted that theoretical work is still needed to assess the range of magnitudes and durations of bottlenecks that make purging likely after introduction from a large equilibrium population. Indeed, up to now, theoretical studies investigating the purging of recessive mutations have mainly focused on mutation-selection-drift equilibrium populations.

To test the premise that purging might have occurred during the invasion of HA, we brought six natural field populations into the laboratory and compared their fitness (Figure 1 and Supplemental Experimental Procedures) under two contrasting levels of consanguinity (inbred versus outbred). By using three replicate populations from both the native and the invasive ranges, we could evaluate differences between the ranges robustly, providing a potent test of how the response to inbreeding is affected by population status (native versus invasive; see Supplemental Experimental Procedures). We measured two traits clearly linked to fitness: generation time and lifetime performance. Generation time is an important trait to examine with respect to invasions because a shorter generation time leads to faster population growth [15]. Our measure of lifetime performance accounts for both survival through the life stages and subsequent reproduction. It thus represents individual fitness well, and it is independent of generation time (Supplemental Experimental Procedures).

We found that the generation time of invasive populations is on average 6.3 days shorter than that of native populations ($p = 0.0005$). Invasion status and level of consanguinity interact ($p = 0.047$; Figure 3 and Table S1) such that the difference is most apparent in inbred individuals. Native populations suffer strong inbreeding depression with respect to generation time (coefficient of inbreeding depression, $\delta = 0.21$, $p = 0.03$), whereas invasive populations suffer none ($\delta = -0.05$, $p = 0.57$) and are thus able to maintain the outbred phenotype. A significant population effect nested within the origin effect (Table S1) reveals that one native population (Abakan, Russia) exhibits a longer generation time in outbred treatment than the other native populations, implying no significant inbreeding depression for this trait in this population. As for generation time, invasive populations have higher average lifetime performance than native ones ($p = 0.02$), and there is a strong interaction between invasion status and level of consanguinity ($p = 0.001$; Figure 3 and Table S1). In general, native populations suffer intense inbreeding depression ($\delta = 0.59$, $p < 0.001$), whereas invasive populations do not ($\delta = 0.12$, $p = 0.16$).

For both traits, invasive individuals exhibit a decline in inbreeding depression and are thus able to maintain the high performance of the outbred phenotype. Inbred invasive individuals developed more quickly and attained a higher lifetime performance than native ones ($p = 0.0005$ and 0.0057, respectively), indicating that inbreeding depression decreased within invasive populations, which is consistent with the predicted purging of recessive deleterious mutations. Moreover, inbred lines from invasive populations developed just as quickly and attained just as high lifetime performance as outbred lines from both invasive and native populations (Figure 3). Purging leads to an overall increase in performance of the invasive populations for these two traits closely linked to fitness, and it might thus have boosted the invasiveness of HA. Indeed, by shortening average generation time and increasing average lifetime performance, the drop in inbreeding depression might increase the population growth rate of invasive populations. Our two main results, evidence of a type of bottleneck consistent with the purging of alleles that lead to inbreeding depressions (i.e., a bottleneck of intermediate intensity) and evidence of such purging in two fitness-related traits, together match the theoretical expectations well. Moreover, theory [3, 5] illustrates that the greatest purging occurs when inbreeding depression is mainly due to mutations that are both strongly deleterious and highly recessive, suggesting that inbreeding depression in native populations of HA probably stems from highly recessive and strongly deleterious mutations.

Several theoretical [3, 16] and empirical [8, 17, 18] studies establish that consanguineous mating increases the efficiency of purging. Geographical spread during the invasion process

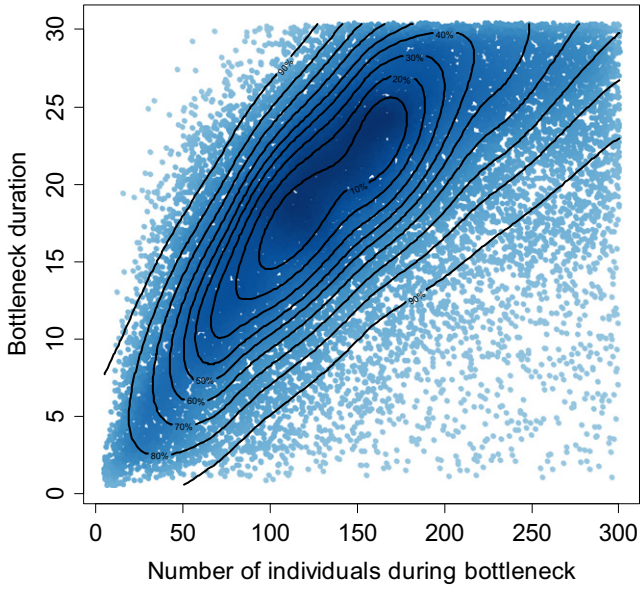

Figure 2. Intensity of the Bottleneck Event Following the Introduction of *Harmonia axyridis* in Eastern North America from Its Native Area

The joint densities of posterior distributions for the correlated pair of demographic parameters number of individuals during bottleneck and bottleneck duration (in number of generations) were obtained via ABC analysis of microsatellite data under the introduction scenario 1 (Figure S1); prior set 1 was assumed (Table S1), and population samples were from Kyoto (Japan) and Brookings (South Dakota, USA), taken as representative of the native and introduced areas, respectively (i.e., the samples were from the same populations as those used for the experiment). See Experimental Procedures for justification of population sampling and Figure S2 for complementary results obtained with different priors and sampling combinations. The black lines represent the 10%–90% highest density contours of the plot of joint densities. Median value of the stable effective population size before and after the bottleneck period was estimated at 2940 individuals (95% confidence interval: 1220 – 8930). See also Figure S1.

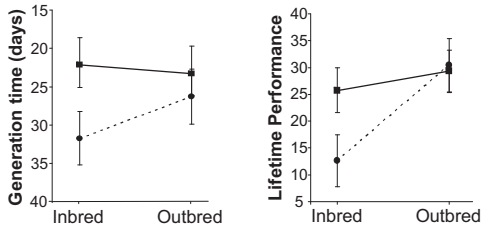

Figure 3. Generation Time and Lifetime Performance of Native versus Invasive Populations and Consanguinity of Inbred versus Outbred Populations

Circles represent native populations, and squares represent invasive ones. Note that the *y* axis shows low values of generation time, which correspond to high fitness, at the top, and high values of generation time (low fitness) at the bottom. Mean values are ±1.96 standard error. See also Table S1.

can promote consanguineous mating in the invasion front. Density in the front can be very low [19], setting up a situation in which individuals from the same clutch have only each other to mate with. In this scenario, purging could be further facilitated in invasive populations and could occur for a broader range of population sizes and in populations with less strongly recessive deleterious mutations.

The invasive populations used in our study are connected by their recent history [12]: the eastern North American invasive population is the main source of the South African and European invasive populations. It is hence probable that the reduction in inbreeding depression evolved only once, in eastern North America, and was subsequently transmitted to the other invasive populations. This mechanism could be responsible for the North American's status as an invasive bridgehead. Because we obviously could not sample all locations within the native range of HA, we cannot completely reject the hypothesis that purging of deleterious alleles occurred within the native range in an unknown way. The hypothesis that purging occurred in the introduced range during the bottleneck period is nevertheless far more parsimonious.

Our results shed new light on four patterns commonly observed in biological invasions. First, they help explain how non-native species spread so rapidly when they become invasive. Even small populations on the invasion front, in which consanguineous matings are probable, can grow quickly without being slowed by inbreeding depression if recessive deleterious alleles have been purged. Second, our findings might explain the "lag time" of invasions: the period of time that is often observed between initial introductions and subsequent invasions [20]. This lag time could be due to negative population growth and initially high rates of local extinction associated with the purging of the deleterious alleles. Once the recessive deleterious alleles are purged, explosive population growth would follow. In HA, it could be that a high rate of extinction of inbred populations contributed to the repeated failures of efforts to establish populations for biological control. Third, our results might explain the finding that invasive populations often have higher performance than native ones even when reared in a common environment. This has been attributed mainly to adaptation to the new range [21–23]. However, a purging of inbreeding depression could explain, at least partly, the increase in performance without invoking local adaptation. This mechanism could be particularly appropriate when there is no obvious adaptive challenge associated with the new introduced environment, as suspected for HA. Finally, a shift toward selfing has been observed in some invasive plants [24, 25]. Inbreeding depression is considered to be one of the main forces opposing the evolution of self-fertilization [26]. A reduction in genetic load during invasions could thus promote a shift from outcrossing toward selfing in invasive plant populations.

Our results link, for the first time in natural populations, bottlenecks of intermediate size during invasion with purging of deleterious mutations. This purging results in the evolution of populations that experience no inbreeding depression in important fitness traits and leads to higher mean fitness relative to native populations. Thus, not only might bottlenecks not pose the problems previously assumed for invasive species [27], but they might actually facilitate invasion. This kind of purging should be particularly important during the first stages of the invasion (when there is a small effective population size) and during the spatial expansion (at the front of invasion), i.e., when mating between relatives is likely to occur most frequently. After this stage, when invasive populations reach a large, stable effective population size, it might be that new deleterious mutations start to accumulate, and thus inbreeding depression might return to invasive populations.

Experimental Procedures

Biological Material

Three native populations (Kyoto in Japan [KYO], Novosibirsk [NOV], and Abakan [ABA] in Russia) and three invasive populations (Croix [FRA] in

France, Brookings [DAK] in South Dakota-USA, and Bethlehem [SAF] in South Africa) were sampled in the wild between 2007 and 2008. The locations were chosen because they cover major parts of the current native and introduced distribution of *H. axyridis* (Figure 1), and the native range populations are within the region likely to have been the source of the invasion [12]. In each population, 80–100 adults were collected. See Supplemental Information for further details on sampled populations.

Inferences about the Bottleneck Event

Two field-collected samples, Kyoto and Brookings, representing the native and bridgehead invasive populations, respectively, were genotyped at 18 microsatellite markers. Using approximate Bayesian computation (ABC), we analyzed two competing introduction scenarios that differed by the presence or absence of a bottleneck event after introduction. We assessed the robustness of our ABC inferences by considering two different sets of prior distributions and by processing our analyses on a second microsatellite data set that included other representative population samples (see Supplemental Experimental Procedures).

Quantitative Genetic Experiment

For each of the six populations sampled, 100 field-collected (G_0) individuals initiated populations in the laboratory. We maintained these populations for two generations under strictly controlled conditions to minimize potential biases due to maternal effects (see Supplemental Information). We then created two types of crosses: inbred (between pairs of siblings) and outbred (between unrelated individuals of the same population). For the two types of G_3 individuals produced, we measured hatching rate, larval survival, development time, time to sexual maturity, and fecundity. Finally, we analyzed two combined traits linked to fitness: generation time and lifetime performance (see Supplemental Experimental Procedure). To calculate generation time, we added egg-to-adult development time and time to reach sexual maturity into a single cumulative measure. We obtained a measure of lifetime performance by multiplying hatching rate by larval survival by subsequent fecundity for each family and cross. We analyzed these data by using mixed-model ANOVAs. Origin (invasive versus native), treatment (inbred versus outbred), population nested in origin, and their interactions were entered as fixed effects. Family nested within population was treated as a random effect.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, one figure, and one table and can be found with this article online at doi:10.1016/j.cub.2011.01.068.

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